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ORIGINAL PAPER

Genetic differentiation between Arctic and Antarctic monothalamous foraminiferans

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Abstract Monothalamous (single-chambered) foraminifers are a major component of the benthic meiofauna in high latitude regions. Several morphologically similar species are common in the Arctic and Antarctic. However, it is uncertain whether these morphospecies are genetically identical, or whether their accurate identification is compromised by a lack of distinctive morphological features. To determine the relationship between Arctic and Antarctic species, we have compared SSU rDNA sequences of specimens belonging to four morphotaxa: *Micrometula*, *Psammophaga*, *Gloiogullmia*, and one morphospecies *Hippocrepinella hirudinea* from western Svalbard (Arctic) and McMurdo Sound (Antarctic). Wherever possible, we include in our analyses representatives of these taxa from the deep Arctic and Southern Oceans, as well as from Northern European fjords. We found that in all cases, the bipolar populations were clearly distinct genetically. As expected, Arctic specimens were usually more closely related to those from Northern Europe than to their Antarctic representatives. The deep-sea specimens from Weddell Sea branched as a sister to the McMurdo Sound population, while those from the Arctic Ocean clustered with ones from Norwegian fjords. Our study has revealed a high number of cryptic species within each of the examined genera, and demonstrates the unexplored potential of monothalamous foraminifers for use as a tool to evaluate the origin and biogeography of polar meiofauna.

Keywords Foraminifera · Bipolar distribution · SSU rDNA · Molecular diversity · Protists

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Introduction

The capacity for dispersion of small organisms such as protists is the subject of active debate. Some recent molecular studies have shown a considerable capacity for dispersion among marine protists (Šlapeta et al. 2006; Pawlowski et al. 2007), in agreement with the hypothesis that microbial species are ubiquitously dispersed (Finlay 2002). In principle, this is congruent with the observed bipolar distribution of many polar and subpolar protists. For example, 44 morphospecies of limnetic ciliates were found to occur in Arctic and Antarctic freshwater bodies (Petz et al. 2007). However, the identification of these and other bipolar species has been based exclusively on morphological characters, which are often inadequate to detect interspecific variations. The few molecular studies of bipolar protists have yielded conflicting results. Arctic and Antarctic populations of the dinoflagellate *Polarella glacialis* (Montresor et al. 2003) and the planktonic foraminiferan *Neogloboquadrina pachyderma* (Darling et al. 2004) have been

shown to differ genetically. On the other hand, molecular analyses of the genus *Naegleria* revealed that two species in Arctic and sub-Antarctic regions were genetically similar (De Jonckheere 2006).

Bipolarity has also been observed in some groups of marine invertebrates, for example, five common genera of amphipods were found in Arctic and Antarctic fjords (Jazdzewski et al. 1995). This pattern of distribution raises several questions. How closely related are members of bipolar species? Does the appearance of bipolarity result from (1) morphological convergence of unrelated species, (2) independent evolution of cold-adapted populations from a common temperate/subpolar stock, or (3) from direct colonization of one polar region by organisms from the other? Do bipolar morphospecies comprise genetically distinct taxa, or is there continuous gene flow between polar populations?

To answer some of these questions, we analysed the phylogenetic relationships of several apparently bipolar populations of polar benthic foraminifers. We chose the monothalamous foraminifers, which comprise species traditionally placed in the orders Allogromiida and Astrorhizida (Sen Gupta 1999). This group, characterized by single-chambered organic or agglutinated tests, played a key role in the early evolution of Foraminifera (Pawlowski et al. 2003). Monothalamous foraminifers are particularly abundant in high-latitude settings, in both deep and shallow waters (Gooday 2002; Korsun 2002). Molecular analyses revealed an extraordinary diversity of these organisms in McMurdo Sound (Pawlowski et al. 2002a) and under the Ross Ice Shelf (Pawlowski et al. 2005). Many new morphotypes have been identified in McMurdo Sound (Gooday et al. 1996) and in Svalbard (Gooday et al. 2005; Majewski et al. 2005). Several of these morphotypes are very similar between the two-polar regions, and the same names are sometimes applied to their identification.

Here, we present genetic analyses of specimens from Arctic and Antarctic populations belonging to four monothalamous morphotaxa (the genera *Micrometula*, *Psammophaga*, and *Gloiogullmia*) and the species *Hippocrepinella hirudinea* (Figs. 1, 2) Our study, based on analyses of partial SSU rDNA sequences, reveals substantial cryptic diversity within each of these morphotypes, and shows that Arctic and Antarctic isolates differ genetically and probably represent distinct species.

Material and methods

Sampling and molecular study

Several specimens of each morphotaxon were collected in Western Svalbard (Arctic) and McMurdo Sound (Antarctic). In some cases, shallow-water populations were also sampled in Kosterfjord (Sweden), Skagerrak (Sweden), the Norwegian Sea (Bergen, Norway) and the North Atlantic (Dunstaffnage, Scotland). Deep-sea samples were taken from the Weddell Sea (Southern Ocean) and Fram Strait (Arctic Ocean). The sampling localities and dates are given in Table 1.

The specimens were isolated from the >125 μm sieve fraction of surface sediment samples. They were thoroughly cleaned with a brush in filter-sterilized sea water, measured, and photographed using a Nikon Coolpix digital camera mounted on a dissecting microscope. DNA was usually extracted from single cells, using guanidine lysis buffer. A 3′ fragment of the small subunit ribosomal DNA (SSU rDNA) was amplified using foraminifer-specific primers s14F3 (5′ ACG CA(AC) GTG TGA AAC TTG) and sB (5′ TGA TCC TTC TGC AGG TTC ACC TAC), and was re-amplified with foraminifer-specific primer s14F1 (5′ AAG GGC ACC ACA AGA ACG) and sB. The PCR amplifications, cloning, and sequencing were done as described elsewhere (Pawlowski et al. 2005).

Phylogenetic analyses

The SSU rDNA sequences were manually aligned using Seaview software (Galtier et al. 1996). The number of analysed sites ranges between 828 (*H. hirudinea*) to 883 (*Psammophaga* sp.). The trees of *Micrometula*, *Gloiogullmia* and *H. hirudinea* were rooted with *Bathysiphon flexilis*, *Pilulina argentea* and *Hippocrepina crassa*, respectively. In the case of polar *Psammophaga*, our tree includes *Psammophaga cf. P. simplora*, *Allogromia crystalllifera*, and two undetermined psammophagids. Distance trees were inferred by the neighbor-joining (NJ) method, with distances corrected using the HKY model of substitution. Phylo_win (Galtier et al. 1996) was used for distance computations, tree building, and sequence divergence calculation, whereas PhyML (Guindon and Gascuel 2003) was used to infer the maximum likelihood (ML) trees, with HKY as the model of evolution, and the Ts/Tv ratio estimated from the data. Bootstrap support values (BV) were calculated based on 1,000 replicates for NJ and 100 replicates for ML trees.

Results

Micrometula

Morphology

The specimens from Svalbard and McMurdo Sound were very similar to *Micrometula hyalostriata* of Nyholm 1952, the only formally described species in this genus. Like

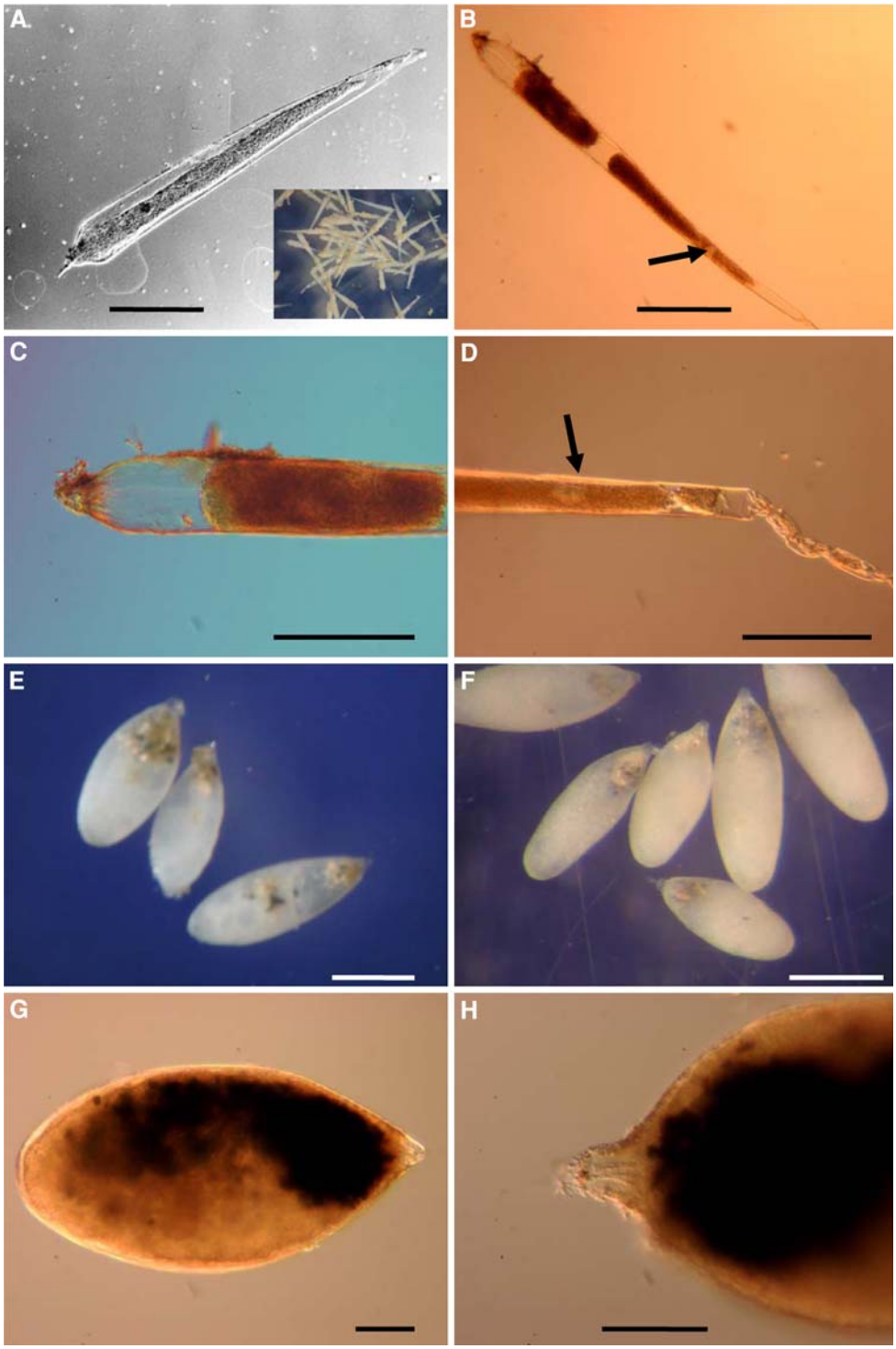

Fig. 1 Light micrographs of Arctic and Antarctic congeners of *Micrometula* sp. (**a**–**d**), and *Psammophaga* sp. (**e**–**h**). **a** Specimen of *Micrometula* sp. from Svalbard (inlet: several specimens from the same area), **b** specimen from Admiralty Bay (Antarctica), an *arrow* indicates the position of the nucleus; **c** higher magnification of the apertural end of the specimen in **b**, showing the striated hyaline theca; **d** higher magnification of the posterior end of another specimen from the same locality, showing the nucleus and the twisted tail; **e** three specimens of *Psammophaga* sp. from Svalbard, with mineral grains at apertural end; **f** six specimens of *Psammophaga* sp. from McMurdo Sound (Antarctica), with mineral grains at apertural end; **g** a specimen of *Psammophaga* sp. from Admiralty Bay (Antarctica), showing the thick organic theca and dark mineral grains filling the cytoplasm; **h** a magnification of apertural end of *Psammophaga* sp. from Admiralty Bay (Antarctica) showing the protruding aperture and dark mineral grains. *Scale bars* 500 μm (**a**), 200 μm (**b**, **e**, **f**), 100 μm (**c**, **d**, **g**, **h**)

M. hyalostriata, the polar specimens of *Micrometula* were characterized by an elongate, conical, organic-walled test with a distinctive aperture at the wider end (Fig. 1a–d). Their tests were of consistent size, that is, up to 1 mm long and about 0.08–0.1 mm wide. A second aperture at the narrower (proximal) end was sometimes observed in the Antarctic *Micrometula* (Gooday et al. 1996) but, as pointed out by Nyholm (1952), this feature could be temporary, or could perhaps result from breakage of the proximal extremity. After collection, living specimens of this genus are often found attached to the bottom of the Petri dish by the main aperture, but we have not observed them to collect food or debris around the proximal end. The longitudinal striations of the test mentioned in the original description

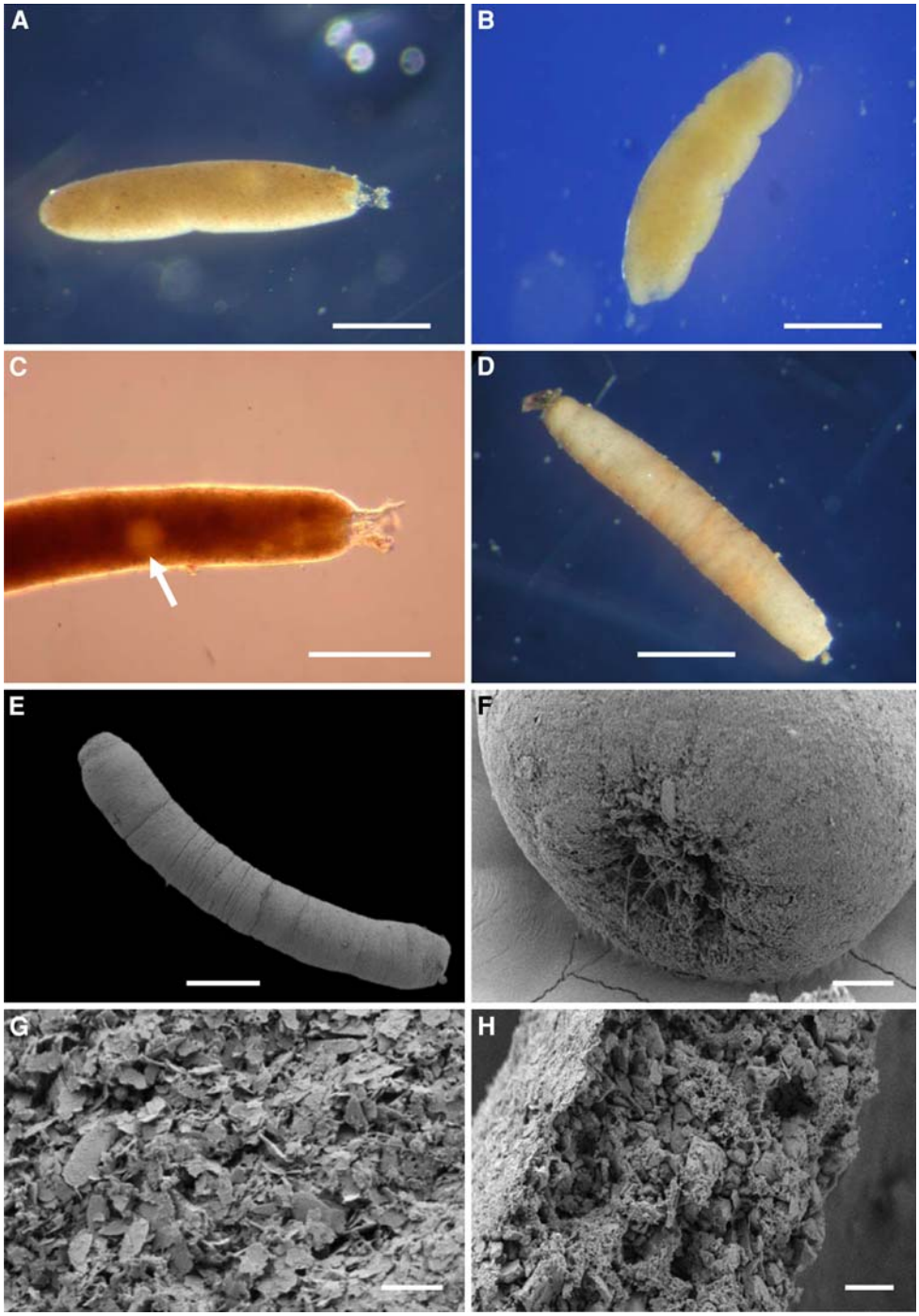

Fig. 2 Light micrographs of *Gloiogullmia* (**a**–**c**) and scanning electron micrographs of *Hippocrepinella hirudinea* (**d**–**h**) from polar regions. **a** A specimen of *Gloiogullmia* sp. from Svalbard; **b** a specimen of *Gloiogullmia* sp. from McMurdo Sound (Antarctica); **c** apertural end of *Gloiogullmia* sp. from Admiralty Bay (Antarctica) with an *arrow* indicating the position of the nucleus; **d** a specimen of *H. hirudinea* from Svalbard; **e** SEM overview of a specimen of *H. hirudinea* from Admiralty Bay (Antarctica); **f** apertural view of the same specimen; **g** high-magnification view of the outer surface of the wall showing tiny mica plates; **h** a cross-section view of the test wall of the same specimen. *Scale bars* 200 μm (**a**, **b**), 100 μm (**c**–**e**), 10 μm (**f**), 1 μm (**g**), 2 μm (**h**)

have been observed only in specimens examined with light microscope (Fig. 1c).

Distribution

Micrometula hyalostriata was originally isolated from soft-bottom regions in the Gullmar Fjord on the west coast of Sweden. The genus is common along the coasts of Sweden, Norway, and Scotland at depths of about 100 m, but it seems to be adapted to a very broad bathymetric range: we have found it at depths of 1,077 and 1,279 m in the Arctic Ocean, at 4,850 m in the NE Atlantic, and at 6,326 m in the Southern Ocean. In coastal waters, the distribution of *Micrometula* was patchy. In Svalbard, the genus was particularly abundant in Adventsfjord at a depth of 70 m, in Kongsfjord at 115 m (Majewski et al. 2005), and in Tempelfjord at 92 m (Gooday et al. 2005). In McMurdo Sound, it was found at localities near McMurdo Station, at Gneiss Point, and at Terra Nova Bay, but it was rare in Explorers Cove.

Table 1 List of DNA isolates and the collection sites

DNA #	Species	Date	Locality	Latitude	Longitude	Depth
AR5524	*Micrometula* sp.	Sep-2005	Arctic Ocean, st. 277	79°8.00 N	6°05.57 E	1279.2
AR5549	*Micrometula* sp.	Sep-2005	Arctic Ocean, st. 314	81°5.84 N	8°38.02 E	1077.6
AR5570	*Micrometula* sp.	Sep-2005	Arctic Ocean, st. 331	80°9.38 N	3°42.32 E	1612.8
BE6831	*Micrometula hyalostriata*	Oct-2006	Bergen, st.6	60°10.843 N	5°14.641 E	659
BE6832	*Micrometula hyalostriata*	Oct-2006	Bergen, st.6	60°10.843 N	5°14.641 E	659
BE6833	*Micrometula hyalostriata*	Oct-2006	Bergen, st.6	60°10.843 N	5°14.641 E	659
BE6844	*Micrometula hyalostriata*	Oct-2006	Bergen, st.7	60°18.214 N	5°11.629 E	148
BE6845	*Micrometula hyalostriata*	Oct-2006	Bergen, st.7	60°18.214 N	5°11.629 E	148
BE6846	*Micrometula* sp.	Oct-2006	Bergen, st.7	60°18.214 N	5°11.629 E	148
DN3974	*Micrometula hyalostriata*	Jun-2003	Creag's Hole, Dunstaffnage	56°28.078 N	5°30.399 W	60
DN3998	*Micrometula hyalostriata*	Jun-2003	Creag's Hole, Dunstaffnage	56°28.078 N	5°30.399 W	60
DN4012	*Micrometula hyalostriata*	Jun-2003	Creag's Hole, Dunstaffnage	56°28.078 N	5°30.399 W	60
MM3226	*Micrometula* sp.	Nov-2001	McM Sound, Herbertson Glacier	77°41.715 S	163°54.642 E	27
MM2920	*Micrometula* sp.	Nov-2001	McM Sound, Herbertson Glacier	77°41.715 S	163°54.642 E	27
MM3217	*Micrometula* sp.	Nov-2001	McM Sound, Herbertson Glacier	77°41.715 S	163°54.642 E	27
MM2939	*Micrometula* sp.	Nov-2001	McMurdo Sound, McM station	77°51.714 S	166°65.903 E	23.6
MM2940	*Micrometula* sp.	Nov-2001	McMurdo Sound, McM station	77°51.714 S	166°65.903 E	23.6
MM2966	*Micrometula* sp.	Nov-2001	McMurdo Sound, McM station	77°51.714 S	166°65.903 E	23.6
MM3190	*Micrometula* sp.	Nov-2001	McMurdo Sound, Gneiss Point	77°23.587 S	163°39.155 E	23
SK6733	*Micrometula hyalostriata*	Aug-2006	Skagerrak, st.47	57°58.996 N	11°10.944 E	104
SK6735	*Micrometula hyalostriata*	Aug-2006	Skagerrak, st.47	57°58.996 N	11°10.944 E	104
SK6735	*Micrometula hyalostriata*	Aug-2006	Skagerrak, st.47	57°58.996 N	11°10.944 E	104
SK6737	*Micrometula hyalostriata*	Aug-2006	Skagerrak, st.47	57°58.996 N	11°10.944 E	104
SV4518	*Micrometula* sp.	Jul-2004	Svalbard, Kongsfjord 4	78°92.09 N	12°26.40 W	110
SV4683	*Micrometula* sp.	Jul-2004	Svalbard, Kongsfjord 7	78°91.80 N	12°23.56 W	115
SV4684	*Micrometula* sp.	Jul-2004	Svalbard, Kongsfjord 7	78°91.80 N	12°23.56 W	115
SV4747	*Micrometula* sp.	Jul-2004	Svalbard, Adventfjord 1	78°24.01 N	15°61.12 W	70
SV4888	*Micrometula* sp.	Jul-2004	Svalbard, Adventfjord (dive)	78°25.29 N	15°46.60 W	6
TB3788	*Micrometula* sp.	Jan-2003	Terranova Bay, Ross Sea	74°40.28 S	164°04.11 E	25
TB3789	*Micrometula* sp.	Jan-2003	Terranova Bay, Ross Sea	74°40.28 S	164°04.11 E	25
WS3549	*Micrometula* sp.	Jan-2002	Weddell Sea st. 142	58°50.76 S	23°58.48 W	6,329
DN3995	*Psammophaga* sp.	Jun-2003	Dunstaffnage—dive			30
DN4020	*Psammophaga* sp.	Jun-2003	Creag's Hole, Dunstaffnage	56°28.08 N	5°30.39 W	60
DN4023	*Psammophaga* sp.	Jun-2003	Dunstaffnage st.3	56°29.25 N	5°28.97 W	46
NH2112	*Psammophaga* sp.	Nov-1999	New Harbor, Tile Hole	77°34.278 S	163°30.117 E	22
NH2114	*Psammophaga* sp.	Nov-1999	New Harbor, Tile Hole	77°34.278 S	163°30.117 E	22
NH3184	*Psammophaga* sp.	Nov-2001	McMurdo Sound, Gneiss Point	77°23.587 S	163°39.155 E	22
SV2897	*Psammophaga* sp.	Aug-2001	Svalbard, Kongsfjord 77	78°55.19 N	12°15.03 E	106
SV4511	*Psammophaga* sp.	Jul-2004	Svalvard, Kongsfjord 4	78°92.09 N	12°26.40 E	110
SV4512	*Psammophaga* sp.	Jul-2004	Svalvard, Kongsfjord 4	78°92.09 N	12°26.40 E	110
SV4513	*Psammophaga* sp.	Jul-2004	Svalvard, Kongsfjord 4	78°92.09 N	12°26.40 E	110
SV4514	*Psammophaga* sp.	Jul-2004	Svalvard, Kongsfjord 4	78°92.09 N	12°26.40 E	110
SV4791	*Psammophaga* sp.	Jul-2004	Svalbard, Adventfjord 3	78°25.60 N	15°58.62 E	80
SV4792	*Psammophaga* sp.	Jul-2004	Svalbard, Adventfjord 3	78°25.60 N	15°58.62 E	80
SV5321	*Psammophaga* sp.	Aug-2005	Svalbard, Storfjord 202	77°34.61 N	20°33.82 E	69
TB3790	*Psammophaga* sp.	Jan-2003	Terranova Bay	74°40.28 S	164°04.11 E	25
DN4001	*Gloiogullmia* sp.	Jun-2003	Off Ganavan, Dunstaffnage	56°26.71 N	5°28.89 W	50
DN4013	*Gloiogullmia* sp.	Jun-2003	Off Ganavan, Dunstaffnage	56°26.71 N	5°28.89 W	50

Table 1 continued

DNA #	Species	Date	Locality	Latitude	Longitude	Depth
NH1188	*Gloiogullmia* sp.	Nov-1998	New Habor—dive hut	77°34.552 S	163°31.742 E	28
NH1828	*Gloiogullmia* sp.	Nov-1999	New Harbor—dive hut	77°34.337 S	163°31.433 E	28
NH1829	*Gloiogullmia* sp.	Nov-1999	New Harbor—dive hut	77°34.337 S	163°31.433 E	28
NH1918	*Gloiogullmia* sp.	Nov-1999	New Harbor—Delta	77°34.335 S	163°31.16 E	20
NH1958	*Gloiogullmia* sp.	Nov-1999	New Harbor—dive hut	77°34.337 S	163°31.433 E	28
SV2882	*Gloiogullmia eurystoma*	Aug-2001	Svalbard, Tempelfjord 57/1	78°56.26 N	17°22.95 E	26
SV2885	*Gloiogullmia eurystoma*	Aug-2001	Svalbard, Tempelfjord 57/1	78°56.26 N	17°22.95 E	26
TJ526	*Gloiogullmia eurystoma*	Sep-1997	Tjärno, Singlefjord	NA	NA	NA
WS3415	*Gloiogullmia* sp.	Apr-2002	Weddell Sea, st 136	64°01.56 S	39°06.94 W	4,749
WS3416	*Gloiogullmia* sp.	Apr-2002	Weddell Sea, st 136	64°01.56 S	39°06.94 W	4,749
MM1157	*Hippocrepinella hirudinea*	Nov-1998	McMurdo Station Jetty	NA	NA	28
MM2973	*Hippocrepinella hirudinea*	Nov-2001	McMurdo Sound, McM station	77°51.714 S	166°65.903 E	23.6
MM2974	*Hippocrepinella hirudinea*	Nov-2001	McMurdo Sound, McM station	77°51.714 S	166°65.903 E	23.6
MM2976	*Hippocrepinella hirudinea*	Nov-2001	McMurdo Sound, McM station	77°51.714 S	166°65.903 E	23.6
MM3009	*Hippocrepinella hirudinea*	Nov-2001	McMurdo Sound, McM station	77°51.714 S	166°65.903 E	23.6
MM3183	*Hippocrepinella hirudinea*	Nov-2001	McMurdo Sound, Gneiss Point	77°23.587 S	163°39.155 E	23
SV2857	*Hippocrepinella* sp.	Aug-2001	Svalbard, Isfjord 80	78°23.39 N	08°44.23 E	1,532
SV2865	*Hippocrepinella* sp.	Aug-2001	Svalbard, Isfjord 80	78°23.39 N	08°44.23 E	1,532
SV4821	*Hippocrepinella* sp.	Aug-2004	Svalbard, Nordfjord 1	78°49.24 N	14°91.76 W	150
TJ530	*Hippocrepinella* sp.	Sep-1997	Tjärno, Singlefjord	NA	NA	NA
Outgroups						
TJ1391	*Allogromia crystallifera*	May-1999	Tjärno, Kosterfjord	NA	NA	NA
TJ2361	*Allogromia crystallifera*	Sep-2000	Tjärno, Singlefjord	NA	NA	NA
DN4004	*Bathysiphon flexilis*	Jun-2003	Off Ganavan, Dunstaffnage	56°26.79 N	5°29.59 W	34
DN4005	*Bathysiphon flexilis*	Jun-2003	Off Ganavan, Dunstaffnage	56°26.79 N	5°29.59 W	34
GU1784	*Bathysiphon flexilis*	Aug-1999	Gullmardfjord	NA	NA	NA
SV2726	*Hippocrepina crassa*	Aug-2001	Svalbard, Tempelfjord 57/4	78°56.26 N	17°22.95 E	26
SV2840	*Pillulina argentea*	Aug-2001	Svalbard, Tempelfjord 63	78°21.58 N	16°49.55 E	80
SV2836	*Pillulina argentea*	Aug-2001	Svalbard, Tempelfjord 57	78°56.26 N	17°22.95 E	26
SV2837	*Pillulina argentea*	Aug-2001	Svalbard, Tempelfjord 57	78°56.26 N	17°22.95 E	26
SV2841	*Pillulina argentea*	Aug-2001	Svalbard, Tempelfjord 57	78°56.26 N	17°22.95 E	26
231	*Psammophaga simplora*	Jun-1996	Sapelo, Georgia, USA	NA	NA	NA
2359	*Psammophaga* sp.	Sep-2000	Hamble River, Southampton	NA	NA	NA
2447	*Psammophaga* sp.	Jan-2001	Playa Bailene, Cuba	NA	NA	NA

Molecular data

We obtained 44 sequences from 31 individuals of *Micrometula*. Phylogenetic analysis of these data reveals six clearly distinctive clades, each one supported by 100% BV (Fig. 3). The individual clades correspond quite well to the geographic areas where the specimens were collected. The basal clade comprises specimens collected in Skagerrak, Bergen, and Oban. This clade may correspond to the originally described species *M. hyalostriata*, although specimens from the type locality (Gullmar Fjord) were not examined in our study. The *Micrometula* species from Svalbard branches between *M. hyalostriata* and the crown clade; the latter is a clade that comprises both the Antarctic specimens from McMurdo Sound and the Weddell Sea and specimens from the Arctic Ocean and Norwegian Sea (Bergen). The resolution in this part of the ML tree is not very high (62% BV), and the distance analyses show Weddell Sea specimens weakly (47% BV) grouping with specimens from the Arctic and Bergen (data not shown). Nevertheless, the sequence divergence among different isolates in this clade ranges from 1.1 to 1.9%, while the divergence within each clade is lower than 0.5%. In comparison, *Micrometula* isolates from McMurdo Sound and Svalbard differ by 3.8–3.9%, indicating that the two populations are genetically distinct.

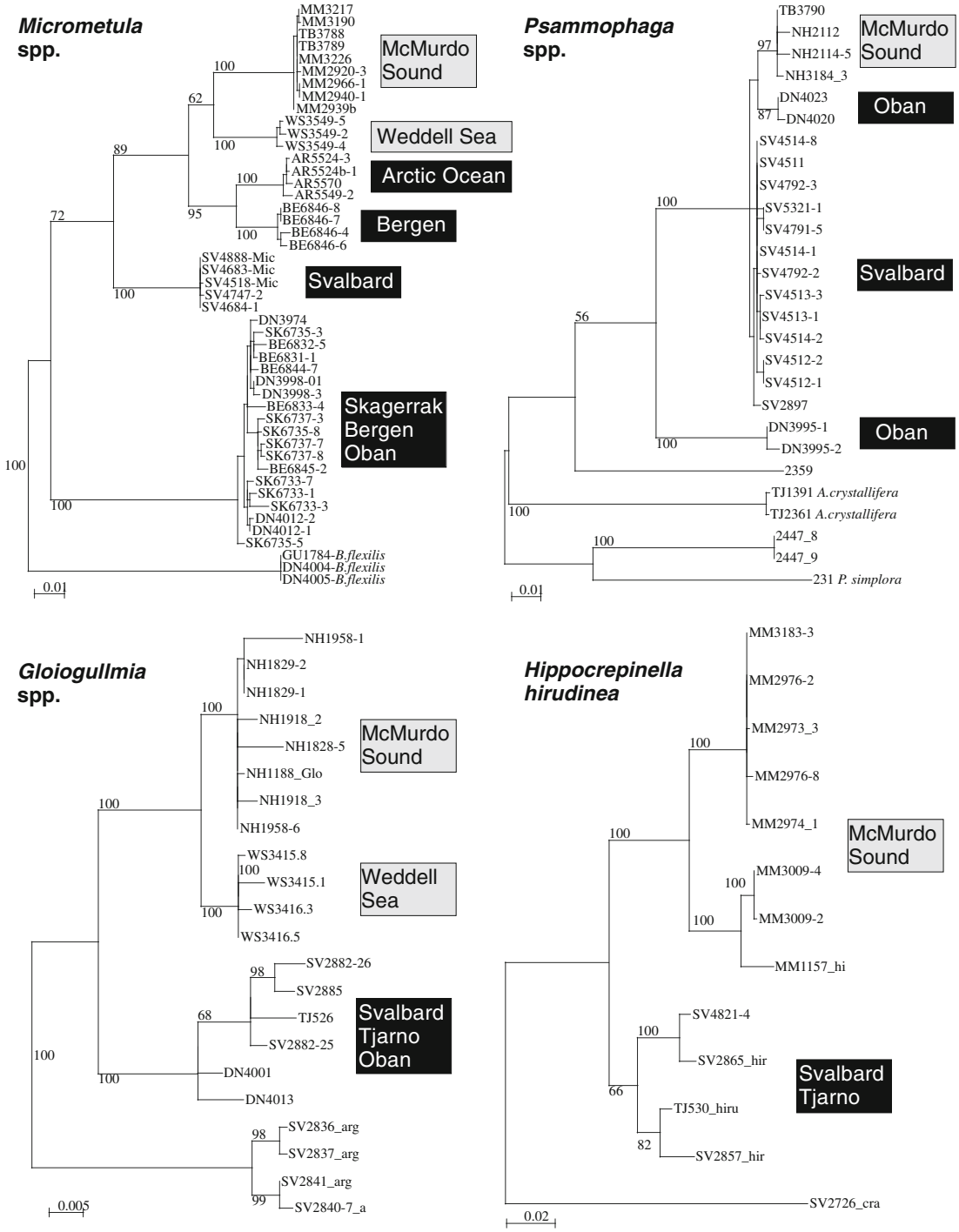

Fig. 3 Phylogenetic tree of Arctic and Antarctic congeners of unilocular foraminiferans inferred from the s14F1-sB fragment of the SSU rDNA. Sequence names reflect DNA isolates identification numbers and clones number, with collection locality given to the right of each clade. *Numbers* above branches are bootstrap support values (>50%)

Psammophaga spp.

Morphology

Like *Micrometula*, the genus *Psammophaga* is currently represented by a single species, *Psammophaga simplora* Arnold 1982, described from shallow waters of Monterey Bay, California. Its most distinctive feature is the presence of mineral particles inside the test. The only other foraminiferal species known to bear similar mineral particles is *Allogromia crystallifera* from Gullmard Fjord (Dahlgren 1962). Although classified in different genera, *Psammophaga*

cf P. simplora and *A. crystallifera* belong to a single clade of monothalamous foraminifers, according to molecular analyses (Pawlowski et al. 2002b); they are therefore grouped together in our analyses (Fig. 3).

The psammophagids from Svalbard and McMurdo Sound are morphologically different from those described by Arnold (1982) and Dahlgren (1962). Their tests have an elongate oval outline and a short apertural tube (Fig. 1e–h), while those of *Psammophaga cf P. simplora* and *A. crystallifera* are typically pyriform or ovoid, with the aperture at the pointed extremity of the test. The wall of *A. crystallifera* is organic, while it is finely agglutinated in *Psammophaga cf P. simplora*. In contrast, the wall of polar psammophagid tests is heterogeneous in appearance. In Svalbard, Gooday et al. (2005) distinguished three morphotypes of *Psammophaga*. Form A has a clearly agglutinated test, while form B has a transparent and predominantly organic-walled test. Gooday's form D resembles form A, but has no inclusions. Forms A and B are 0.24–0.46 mm long and 0.06–0.14 mm wide. Based on different samples from Svalbard, Majewski et al. (2005) distinguished four morphotypes of *Psammophaga* (sp. 1–sp. 4), splitting the organic-walled form B into species 1 and 2, which differ by the quantity of intracellular mineral particles. Only one of these morphotypes (form A) was found in McMurdo Sound (Gooday et al. 1996).

Distribution

Unlike *Micrometula*, which was found in both shallow and deep-sea samples, the psammophagids seem to be restricted to shallow water. They were abundant in Svalbard fjords (Majewski et al. 2005) and common in Explorers Cove, Antarctica, but have not been found in the deep Arctic and Southern Oceans. In addition to specimens from polar regions and from Scotland, our analyses included *A. crystallifera* from Kosterfjord, a site close to the type locality, as well as an isolate of *Psammophaga cf P. simplora* from Sapelo Island (Georgia, USA), which seems to be similar morphologically to the type species from Monterey Bay (S. Goldstein, personal communication). To these, we added sequences of specimens from Cuba (Playa Bailene) and England (Southampton) (Larkin and Gooday 2004). This genus is also known from coastal habitats in the Black Sea (Anikeeya 2005).

Molecular data

In total, 27 sequences from various psammophagids were analysed, including four sequences from McMurdo Sound and 13 sequences from Svalbard (Kongsfjord, Adventfjord and Storfjord). All sequences from McMurdo Sound and Svalbard group into a strongly supported (100% BV) clade (Fig. 3). This "polar" clade also contains two sequences from Scotland, but their position within the clade is not well defined. The sequence divergence within this "polar"clade is very low (0.7–1.3%), with divergence within Antarctic and Svalbard isolates of less than 0.79 and 0.57%, respectively. The sister to this clade is a *Psammophaga*-like isolate from Oban. Although this sister-group relationship was found in both the ML and NJ trees, it is rather weakly supported (56 and 74% BV, respectively). Relationships at the base of the psammophagid tree, between *Psammophaga cf P. simplora*, *A. crystallifera* and other isolates, are similarly not resolved and vary depending on the method of analysis.

Gloiogullmia spp.

Morphology

The genus *Gloiogullmia* is characterized by a sticky (Gr. *gloios*, glue) outer test surface. The only formally described species, *Gloiogullmia eurystoma* Nyholm 1974, was originally reported from the Gullmar Fjord and is characterized by a large aperture and an elongate ovoid test up to 2 mm in length. Specimens of this morphotype collected in Svalbard and McMurdo Sound were also sticky; they often adhered to the brush and were difficult to clean. The test is more or less elongate, sometimes slightly curved, with a single terminal aperture at the end of a short neck (Fig. 2a–c). The cytoplasm is yellow or greenish. Specimens from Svalbard were more elongate than those from McMurdo Sound, but the test morphology was quite variable at both localities. In contrast, the two *Gloiogullmia* morphotypes from the Weddell Sea were much more spherical in shape and were initially misidentified as *Bathyallogromia*.

Distribution

In Svalbard, *Gloiogullmia* was very abundant in some samples; for example, it comprised 41% of monothalamous foraminifers in Adventfjord (Majewski et al. 2005). However, the genus was usually represented by only a few specimens. In McMurdo Sound, *Gloiogullmia* was common in samples from Explorers Cove, but it was quite rare off McMurdo Station. Explorers Cove *Gloiogullmia* were sometimes found inside infaunal tunicates or attached to pycnogonid legs.

Molecular data

We obtained and analysed 18 sequences of *Gloiogullmia* from Svalbard, McMurdo Sound, Oban, and the Weddell Sea. Our analyses revealed three clades corresponding to the geographic origins of the sequenced specimens

(Fig. 3). The specimens from McMurdo Sound (which originated exclusively from New Harbor) form a strongly supported (100% BV) clade with specimens from the Weddell Sea, while specimens from Svalbard, together with those from Oban, branch as a sister group to the Antarctic clade. Sequence divergence among the three clades reaches 4.2%, while the divergence within the clades varies from 0.1 to 0.9%. This divergence is due mainly to several repeats in one of the variable regions, which is very difficult to sequence. Because *G. eurystoma* was originally reported from the coast of Sweden, where one of our sequenced specimens was collected, the clade comprising this specimen and isolates from Svalbard and Scotland most likely represent *G. eurystoma*.

Hippocrepinella hirudinea Heron-Allen and Earland 1932

Morphology

Unlike the other three morphotaxa, the genus *Hippocrepinella* is represented by several described species. Among these, *H. hirudinea* has a very characteristic morphology, and thus the species name rather than the genus name is used here. In general appearance, *H. hirudinea* resembles a short stick with rounded ends, each one bearing an aperture, in accord with the original description (Fig. 2d, e). However, in our specimens as well as in those from Sweden examined by Höglund (1947), only one aperture was observed (Fig.2f). The wall of *H. hirudinea* is relatively thick, smooth, and grey in color, covered with tiny mica plates as shown by the SEM pictures (Fig. 2g, h). Fine transverse wrinkles are present on the outside of the test. Specimens are relatively large, up to 2 mm in length and 0.5 mm in width.

Distribution

Hippocrepinella hirudinea was first described from South Georgia Island by Heron-Allen and Earland (1932). The species was common in most of our samples from various localities in McMurdo Sound, but it was much less abundant in Svalbard samples. In Antarctica, it has also been found on the Terra Nova Bay inner shelf (Volanti 1996) and in Admiralty Bay, King George Island (Majewski et al. 2007). It has been reported from Skagerak, Kattegat, and Gullmar Fjord in Sweden (Höglund 1947), as well as from 280 to 1,750 m water depth in the North Atlantic (Thies 1991).

Molecular data

We compared six specimens from McMurdo Sound, three specimens from Svalbard, and one specimen from Sweden. The specimens from McMurdo Sound (almost all collected off McMurdo Station) form a strongly supported (100% BV) clade, split into two subclades (Fig. 3). Sequence divergence within and between these subclades varies from 0.1 to 0.8% and from 2.8 to 3.0%, respectively. Two subclades, which diverge by 3.1–3.7%, are also observed in the Svalbard + Sweden clade. The divergence between Antarctic and Arctic clades reaches 5.9%. A clade of closely related but more divergent sequences was also observed in an environmental DNA survey of McMurdo Sound foraminifers (Habura et al. 2004). We did not include the latter sequences because their morphotypic origins are not known.

Discussion

Bipolarity in question

Our study shows that none of the Arctic and Antarctic populations of the examined monothalamous foraminifers that we examined are genetically identical. For three of the four morphotaxa, the sequence divergence ranges from 3.8 to 5.9%, suggesting that, despite their structural similarities, the specimens from the Arctic and Antarctic populations represent distinct species. The exception is specimens from the genus *Psammophaga*, in which the sequences of Svalbard and Antarctic isolates differ by less than 1%. Such low divergence may be due either to very slow rDNA substitution rates in *Psammophaga*, or else to relatively recent colonization of the polar regions by this genus. Nevertheless, the Antarctic clade of *Psammophaga* is strongly supported in our analyses (97% BV); we can thus in this case discard the possibility of genetic mixing between Arctic and Antarctic populations.

The genetic differentiation revealed by our study provides new evidence that the bipolarity observed in some marine organisms may be an artifact caused by a lack of distinctive morphological characters, or by imprecise species determinations. This is probably the case for most of the invertebrates (K. Jazdzewski, personal communication). As shown by the present and previous studies, DNA sequences provide an extremely efficient tool for testing the relationships among putative bipolar morphospecies, particularly among microbial eukaryotes. Northern and Southern populations of some of these morphospecies, including dinoflagellates (Montresor et al. 2003) and planktonic foraminifers (Darling et al. 2004), have been shown to be genetically distinct. Detailed morphometric studies have also been effective in demonstrating differences between closely similar Arctic and Antarctic morphotypes in the foraminiferal genus *Cornuspiroides* (Schmiedl and Mackensen 1993).

However, other studies demonstrate that the existence of true bipolar microbial species cannot be excluded. Species of free-living amoebae of the genus *Naegleria* with identical ITS2 sequences have been isolated from Arctic and from sub-Antarctic regions (De Jonckheere 2006). Because these amoebae were isolated at room temperature, it is possible that they are representatives of a cold-tolerant temperate-water species, as opposed to true bipolar organisms, but bipolarity cannot be disproved. Genetically similar species of bacteria and archaebacteria have also been found in Arctic and Antarctic oceans (Hollibaugh et al. 2002; Brinckmeyer et al. 2003; Bano et al. 2004). Moreover, a recent study shows that the deep-sea calcareous foraminifers from the Arctic Ocean are genetically similar to those from the Southern Ocean (Pawlowski et al. 2007). In the latter case, however, the examined species are cosmopolitan and their distributions are not restricted to polar regions. It has been proposed that shallow-water foraminiferal species show patterns of genetic differentiation that are distinct from those for deep-water species (Brandt et al. 2007). Whether this holds true for all groups of marine organisms, or only for particular taxa, remains to be determined.

Cryptic diversity

Our data demonstrate not only genetic differentiation between Northern and Southern populations of certain monothalamous foraminifers, but they also reveal the existence of several genetic lineages that differ considerably from one another. At present, three (*Micrometula*, *Psammophaga*, and *Gloiogullmia*) of the four examined morphotaxa are represented by only one formally described species, although in the case of *Psammophaga*, another described species (*A. crystallifera*) clearly belongs in the genus. Two of these species are represented in our analyses by sequences from an area close to the type locality (Skagerak for *M. hyalostriata*, and Kosterfjord for *G. eurystoma*). Our McMurdo Sound sequences are geographically closest to the original type locality for *H. hirudinea*, in the Falkland Islands area of the Southern Ocean (Heron-Allen and Earland 1932). *Psammophaga cf. P.simplora* is represented by sequences from Sapelo Island, Georgia (USA); our Arctic and Antarctic collection sites are approximately equidistant from the type locality.

In addition to four described species, our analyses revealed 12 other phylotypes: five in *Micrometula*, four in *Psammophaga*, two in *Gloiogullmia*, and one equating to *H. hirudinea*. This number could be even higher if we consider separately the three subclades of polar *Psammophaga* and the four subclades of *H. hirudinea*. However, in the case of polar psammophagids, their sequences are very similar (<1%), while the *H. hirudinea* subclades comprise sequences from the same geographic origin. This may indicate unusually high rDNA variation in this species complex, or sympatric speciation among morphologically similar populations.

The high cryptic diversity revealed by our study is not unexpected. Several previous studies have shown that many foraminiferal morphospecies can be split into a number of genetically distinctive phylotypes (de Vargas et al. 1999; Darling et al. 1999; Holzmann 2000). Hidden diversity was found to be particularly important in monothalamous foraminifers (Pawlowski et al. 2002a; Habura et al. 2004), although, none of the cited studies analysed the problem across a geographic range as broad as was the case in the present investigation. Our study shows that each of the new phylotypes has a more or less restricted geographic distribution. This finding indicates that geographic isolation plays an important role in speciation of monothalamous foraminifers, and that each phylotype identified here probably represents a true species. The morphological description of these species, however, could be problematic. In the calcareous genus *Ammonia*, a morphometric analysis based on over 30 morphological features was necessary to distinguish the 12 genetically distinct types (Hayward et al. 2004). To establish that number of defining characters in the morphology of monothalamous species may be quite challenging.

Phylogeography of polar meiofauna

The results of our study suggest that monothalamous foraminifers are potentially useful tools for inferring phylogeographic patterns in high-latitude meiofauna. Some morphotypes are present across a considerable depth range in polar and subpolar areas, and also occur in more temperate waters. Such broad distributions facilitate the phylogeographic analyses of sequence data and provide valuable sources of information on the origin and phylogeny of polar taxa.

Our data indicate that some polar species are derived from subpolar or more temperate stocks. For example, the temperate species *Psammophaga cf P. simplora* and *A. crystallifera* are basal to psammophagids from the Arctic and Antarctic. One isolate from Scotland, however, appears at the base of the "polar" clade, while a second isolate is included within this clade. We observed the same pattern in the genus *Micrometula*: isolates from Sweden, Norway, and Scotland branch as a sister to the clade of polar species, and one population from Norway branches within this clade. The grouping of subpolar isolates of *Psammophaga* and *Micrometula* within clades that also contain polar isolates could indicate that the colonization of Polar Regions occurred more than once; alternatively, these subpolar isolates may represent polar lineages that returned to subpolar and/or temperate regions.

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